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DEPENDENCE OF PHRENIC MOTONEURONE OUTPUT ON THE OSCILLATORY COMPONENT OF ARTERIAL BLOOD GAS COMPOSITION

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SUMMARY

1. The hypothesis that respiratory oscillations of arterial blood gas composition influence ventilation has been examined.

2. Phrenic motoneurone output recorded in the C5 root of the left phrenic nerve and the respiratory oscillations of arterial pH in the right common carotid artery were measured in vagotomized anaesthetized dogs which had been paralysed and artificially ventilated.

3. The effect of a change in tidal volume for one or two breaths on phrenic motoneurone output was measured with the inspiratory pump set at a constant frequency similar to, and in phase with, the animal's own respiratory frequency. A reduction of tidal volume to zero or an increase by 30 % led to a corresponding change of mean carotid artery pH level. The changes of carotid artery pH resulted in a change of phrenic motoneurone output, predominantly of expiratory time (T_e) but to a lesser extent of inspiratory time (T_i) and also peak amplitude of 'integrated' phrenic motoneurone output (*Phr*). Denervation of the carotid bifurcation blocked this response.

4. The onset of movement of the inspiratory pump was triggered by the onset of phrenic motoneurone output. When a time delay was interposed between them, the phase relationship between respiratory oscillations of arterial pH and phrenic motoneurone output altered. The dominant effect was to alter T_e; smaller and less consistent changes of *Phr* and T_i were observed.

5. When the inspiratory pump was maintained at a constant frequency but independent of and slightly different from the animal's own respiratory frequency (as judged by phrenic motoneurone output), the phase relationship between phrenic motoneurone output and the respiratory oscillations of pH changed breath by breath over a sequence of 100–200 breaths, without change of the mean level of arterial blood gas composition. T_e varied by up to 30 % about its mean value depending on the phase relationship. T_i and *Phr* were also dependent on the phase relationship but varied to a lesser extent. The changes were comparable to the results obtained in paragraph 4.

6. It was concluded that phrenic motoneurone output is dependent in part on its relationship to the respiratory oscillations of arterial blood gas composition.

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7. Information concerning a transient ventilatory disturbance is stored in the arterial blood in the form of an altered pattern of the respiratory oscillations of blood gas composition; this in turn can change breathing by an effect on the carotid bodies.

INTRODUCTION

It is now well recognized that the cyclic nature of breathing generates oscillations of arterial blood composition about a mean level (Nims & Marshall, 1938; Honda & Veda, 1961; Purves, 1966; Band, Cameron & Semple, 1969). The question which remains unanswered is whether or not these oscillations can, in turn, affect breathing.

A pathway has been established which can sense the respiratory oscillations of arterial blood gas composition through the carotid bodies (Hornbein, Griffo & Roos, 1961; Biscoe & Purves, 1967; Black, McCloskey & Torrance, 1971). Furthermore, injections of saline equilibrated with CO_2 or with O_2 which produce an abrupt disturbance of arterial blood gas composition at the carotid bodies in the cat, affect ventilation (Black & Torrance, 1967; Band, Cameron & Semple, 1970; Eldridge, 1972*a*, *b*; Eldridge, 1976; Hanson, Nye & Torrance, 1978).

In order to determine whether or not the respiratory oscillations of arterial blood gas composition affect breathing, the oscillating signal has been perturbed either by altering the amplitude of the oscillations or by disturbing their temporal relationship to the ventilatory cycle. Experiments designed to change the amplitude have produced conflicting results (Lamb, 1966; Sylvester, Whipp & Wasserman, 1973; Lewis, 1975; Linton, Miller & Cameron, 1976; Grant & Semple, 1976; Fordyce, Greco, Gonzalez, Reischl & Grodins, 1977; Ponte & Purves, 1978; Stremel, Huntsman, Casaburi, Whip & Wasserman, 1978).

The temporal relationship between the oscillations of blood gas composition and breathing has been altered by delay coils, by cross-circulation experiments in cats and by altering the pattern of alveolar carbon dioxide tension in human subjects (Band, Ebden, Semple & Wolff, 1971; Black & Torrance, 1971; Cunningham, Howson & Pearson, 1973). The evidence from these experiments is that breathing is affected by its temporal relationship to the oscillations of arterial blood gas composition but the nature of the response has not been clearly defined.

The present study employs a quite different technique for determining if the phase relationship between respiratory and blood gas cycles affects breathing. Experiments were conducted in vagotomized, paralysed, anaesthetized dogs ventilated artificially. Bilateral cervical vagotomy enabled the ventilation to be manipulated without inducing changes related to lung volume feedback. By using a ventilator, which could be activated by the animal's own phrenic motoneurone output, it was possible to alter the relationship between the respiratory and blood gas cycles keeping mean blood gas composition constant. In addition the effect of alteration of mean blood gas composition on phrenic motoneurone output was examined by brief disturbances of tidal volume such as might occur in normal breathing. Preliminary communications of these findings have been given elsewhere (Cross, Grant, Guz, Jones & Semple, 1977, 1978).

METHODS

Mongrel dogs weighing between 10 and 18 kg were used. Anaesthesia was induced with sodium thiopentone (I.V. 2·5%, 25 mg.kg^{-1}) and the animals were intubated with an endotracheal tube (size 9–12)). Anaesthesia was maintained with 1–1·5% halothane in a 50% mixture of N_2O and O_2 while surgical procedures were carried out; this anaesthesia was then replaced with chloralose (I.V., Merck, 40 mg.kg^{-1}). Polyvinyl catheters (internal diameter 1·5 mm) were inserted into the femoral artery and vein. The femoral artery catheter was used to obtain blood samples for measuring arterial blood pH, P_{CO_2} and P_{O_2} with a Radiometer ABL 1 and for measuring arterial blood pressure with a Cambridge pressure transducer (PT8). The C5 root of the left phrenic nerve and both cervical vagi were exposed, dissected free of surrounding tissue and cut. The animals were given I.V. heparin (5000 u.) before insertion of a polyethylene loop (mean internal diameter 3 mm) into the right common carotid artery; whole body heparinization was maintained with hourly administration of 2500 u. per dose. The loop had a side arm into which a needle could be inserted containing a fast response pH electrode. This technique for continuous recording of arterial pH has been described elsewhere (Band & Semple, 1967; Cowell, Band & Semple, 1967). The glass electrode fitted closely into the lumen of a short length of needle tubing; blood flowed through a narrow annular space between the electrode and the tubing, through a sidearm on the needle, then through a saturated calomel reference electrode and returned into the femoral vein via the catheter. In some experiments carotid arterial blood flow was measured with a Statham electromagnetic flow probe SP 7515 coupled to a flowmeter SP 2202.

The methods used to record phrenic motoneurone output have been described in detail elsewhere (Bartoli, Cross, Guz, Huszczuk & Jefferies, 1975). After removing the superficial nerve sheath, the cut central end of the exposed C5 phrenic nerve root was placed on silver/silver chloride bipolar electrodes and immersed under paraffin. The action potentials were amplified using a Devices type 3 pre-amplifier oscilloscope unit (80–2500 Hz, 3 db band width). The amplified signal was then electrically processed to give a voltage known to be proportional to transpulmonary pressure. This signal was called the 'integrated' phrenic signal.

When surgical procedures had been completed, the animals were paralysed with I.V. gallamine triethiodide (Flaxedil) 120 mg and attached to a ventilator (Medipan, Warsaw). The ventilator was used to provide a ramp volume input to inflate the lungs, but expiration was passive. The inspired tidal volume, inspiratory time and respiratory frequency could be set independently. The frequency of the ventilator was determined in one of two ways: either the ventilator operated in the 'automatic mode' at a rate independent of the animal's breathing (as judged by the phrenic motoneurone output), or the ventilator operated in the 'triggered mode' activated by the onset of the 'integrated' phrenic signal. This activation was achieved by passing the 'integrated' phrenic signal to a Spike Trigger sensitive to slowly rising wave forms (Type NL200, Neurolog, Digitimer, England). The spike output was converted to a negative-going logic pulse by a converter (Type NL520, Neurolog) and this pulse then started the sweep of a quartz clock-controlled digital instrument (Digitimer Type 3290). A delay could be introduced by the experimenter in the Digitimer before the signal triggered the ventilator; this provided a means of interposing a variable delay between the onset of inspiration and lung inflation (Fig. 1). Without this delay, the onset of inflation began 20–50 msec after the onset of the 'integrated' phrenic signal.

Airflow was measured by a pneumotachograph (Fleisch no. 1) connected to the endotracheal tube; the differential pressure across the pneumotachograph was measured with a Validyne MP45 variable reluctance transducer $\pm$ 2 cm H_2O). Tracheal CO_2 was measured with a Beckman LB2 infra-red analyser. Analogue data were recorded on an eight-channel Brush recorder (Gould Inc., U.S.A.).

Before starting the experimental procedures, any metabolic acidaemia was corrected with an 8·4% solution of sodium bicarbonate. Further doses of sodium bicarbonate, choralose (20 mg.kg^{-1}) and gallamine were given during the experiment as required. A period of not less than 40 min was allowed for the animal to reach a steady state before any experimental manoeuvres were carried out.

The term 'breath' will be used in this paper to refer to a cycle of phrenic motoneurone activity, even though the animal is artificially ventilated.

Transient disturbance of tidal volume

The ventilator was set on the 'automatic mode' with the onset of inflation adjusted to coincide with the onset of phrenic motoneurone output. The tidal volume and inspiratory times were adjusted to give adequate ventilation as judged by blood gas measurement. After a control period of at least five breaths, the inflation volume of the ventilator was either reduced to zero

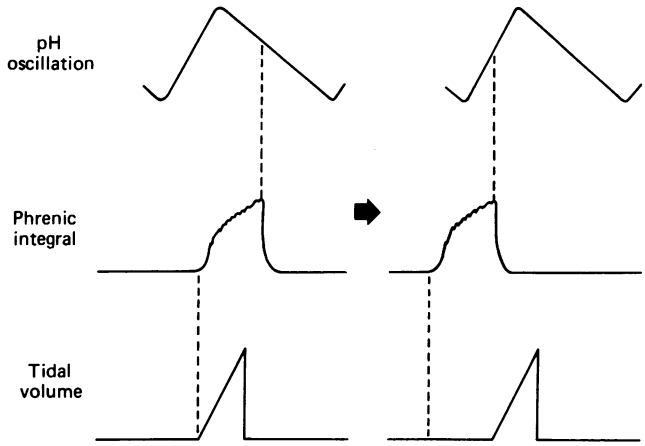

Fig. 1. Schematic representation of how the phase relationship between pH oscillation and phrenic integral is changed by introducing a delay. Left: control situation, no delay. Right: experimental situation with delay. Note that because of the lung-electrode circulation time each pH oscillation shown results from an earlier lung inflation than that indicated.

or increased by about 30 % for one or two cycles; the volume was then returned to the control value for at least a further eight breaths. Each trial was repeated three to eight times. In some experiments transient disturbances of tidal volume were repeated after denervation of the carotid bodies. This was achieved by stripping all the nervous tissue from around the carotid bifurcation and the common carotid artery. In addition, 5 % phenol was injected into the adventitia surrounding the artery, care being taken to avoid injection into the lumen. The expected rise in blood pressure occurred, as anticipated, from the accompanying denervation of the carotid sinus baroceptors. Denervation was judged to be complete when no response was seen on the phrenic motoneurone output to I.V. injection of potassium cyanide (0·5–5 mg) or sodium dithionite (0·5–2·0 ml. of the freshly prepared 0·25 M solution in distilled water, Critchley & Ungar, 1974). The effectiveness of this procedure is shown in Fig. 2.

Alteration of temporal relationship between inflation and phrenic motoneurone output

This procedure was called 'phase shifting' and was accomplished in one of two ways. For the first method the ventilator was set to the 'triggered mode' with the duration of lung inflation matched to the duration of phrenic motoneurone output. After a control period (pre-control) a delay was introduced between the onset of phrenic motoneurone activity and the onset of lung inflation with the Digitimer (Fig. 1). Once a steady state had been reached the delay was removed and the animal allowed to return to control conditions (post-control). This was called a 'stationary phase shift', because the phase relationship between ventilator and phrenic motoneurone activity was maintained constant over a period of time. When the change in phase led to a change in respiratory frequency and hence a change in blood gas tensions, tidal volume was altered manually to hold end-tidal P_{CO_2} (P_{ET,CO_2}) and mean arterial pH constant as judged from the intra-arterial pH electrode record.

For the second method, the ventilator was set on the 'automatic mode' with a frequency slightly different from that of the animal's respiratory frequency. The duration of lung inflation

was similar to the duration of phrenic motoneurone discharge. When the animal was in a steady state a sequence of 50–200 breaths was recorded. The discrepancy between the rate of the ventilator and the respiratory frequency of the animal caused the relationship between lung inflation and phrenic motoneurone activity to change with each breath. A record obtained in one dog is given in Fig. 3; it illustrates the way in which respiratory and blood gas cycles moved in relation to each other.

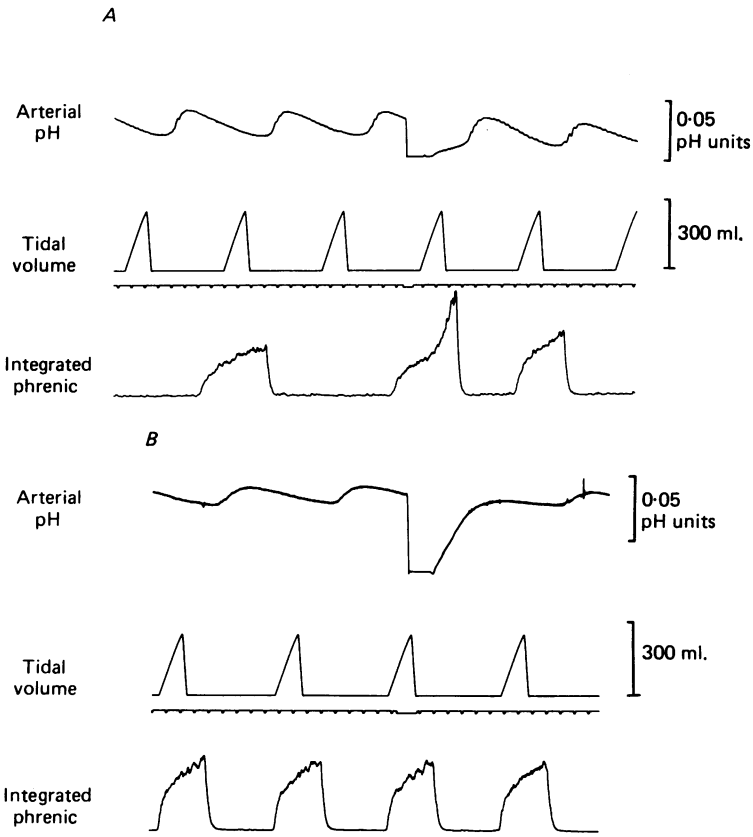

Fig. 2. The effect of an intra-aortic injection of 0·4 ml. 0·25 M-sodium dithionite (*A*) before and (*B*) after carotid body denervation. In both cases the traces from the top are (i) arterial pH, acid direction downwards, (ii) inflation volume, (iii) time trace showing one sec intervals and also event mark at onset of injection and (iv) 'integrated' phrenic signal. Note that in *A* phrenic activity increases approximately 1·5 sec following the sharp acid deflexion in response to the injection; in *B* there is no change in phrenic activity in response to the injection.

In the stationary phase experiments P_{ET,CO_2} and the recording from the intra-arterial pH electrode were used as a guide for maintaining the mean arterial P_{CO_2} and pH constant. In order to determine if P_{ET,CO_2} and the pH electrode were faithfully recording the corresponding changes in arterial blood, a comparison was made in some experiments between these measurements and those made on arterial samples taken simultaneously; the comparison was made before and after experimental manoeuvres in four dogs on a total of eighteen occasions. The mean change in P_{ET,CO_2} was $-0{\cdot}41$, S.D. 1·0 mmHg whilst that of $P_{a.CO_2}$ was $-0{\cdot}54$, S.D. 1·4 mmHg. The corresponding result for mean change in pH from the intra-arterial electrode was $+0{\cdot}003$, S.D. 0·009 and this is to be compared with $+0{\cdot}001$, S.D. 0·009 obtained from the arterial samples.

The following variables were measured from the chart recording: the amplitude of the 'integrated' phrenic signal (*Phr*); inspiratory duration (T_i), defined as the interval between the time when the 'integrated' phrenic signal began to rise to the time when it began to fall; expiratory duration (T_e), defined as the remainder of the respiratory cycle; the duration of the pH cycle (pH_{tot}); the duration of the alkaline tide (pH_{alk}) and the point in the cycle which coincided with the peak of the 'integrated' phrenic signal (pH_t). The numerical value for the phase relationship (ϕ) between phrenic motoneurone output and pH cycle was obtained by expressing pH_t as

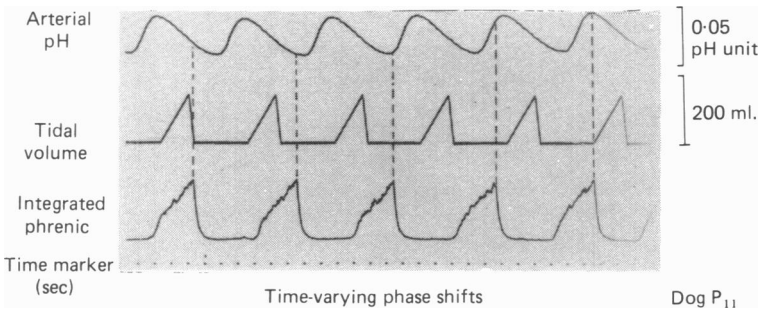

Fig. 3. A record from above downwards of arterial pH, tidal volume and the 'integrated' phrenic signal in a time-varying phase shift experiment. The discrepancy between respiratory rate (judged from the phrenic motoneurone output) and the rate of the ventilator leads to a continuous variation of phase. Time is marked at 1 sec intervals.

a proportion of pH_{tot} (see Fig. 4). All possible values of ϕ lie between zero and unity. Values of 0 and 1 represent the same position in the pH cycle. End-inspiration has been chosen as the point of reference for the phase relationship for two reasons. First, it is the most clearly demarcated point in the respiratory cycle as represented by the phrenic motoneurone output. Secondly, studies on the effect of abrupt stimuli on ventilation (Black & Torrance, 1971) have shown that the latter part of inspiration is a highly sensitive time for these chemical stimuli to be effective.

Phrenic amplitude was measured to an accuracy of 2%, timing to 0·05 sec from records obtained at 5 mm.sec^{-1} and F_{ET,CO_2} to 0·1%.

Statistical analysis

In all analytical techniques, significance was accepted at the 5% level. Linear and polynomial regression analyses were applied as necessary using statistical packages available in the University of London CDC Computer (B. M. D. Dixon, 1971).

Transient disturbance of tidal volume

Each manoeuvre was repeated 3–8 times in succession. Values of *Phr*, T_i and T_e for each experimental breath were expressed as a percentage of the averaged value of the preceding five control breaths in each trial. Data from the individual trials were then pooled in each dog. In this way the first experimental breath of each trial was averaged and the same procedure was then applied to each subsequent breath. Each of these averaged responses was then compared with the pooled data for all control breaths using Student's unpaired *t* test.

Stationary phase shifts

The average value of *Phr*, T_i, T_e and ϕ for five breaths in the pre-control state were compared with corresponding averaged values for five breaths in the post-control state using Student's unpaired *t* test. The data was excluded from further analysis if significant differences of 10% or greater were found. Pre- and post-control data for each variable were pooled and compared with the averaged measurements of *Phr*, T_i, T_e and ϕ for five breaths during the period of phase shifting, again using Student's unpaired *t* test.

Time-varying phase shifts

Values of *Phr*, T_i, T_e and ϕ were obtained for a sequence of 50–200 breaths over a period of 20 min. *Phr*, T_i and T_e were each tested for dependence on ϕ with polynomial regression analysis up to the fifth degree, using an analysis of variance. The graph of the quintic expression was used to define the relationship between the dependent variables and ϕ; this gave the best description of the data in terms of least squares and made it possible to see if there were more

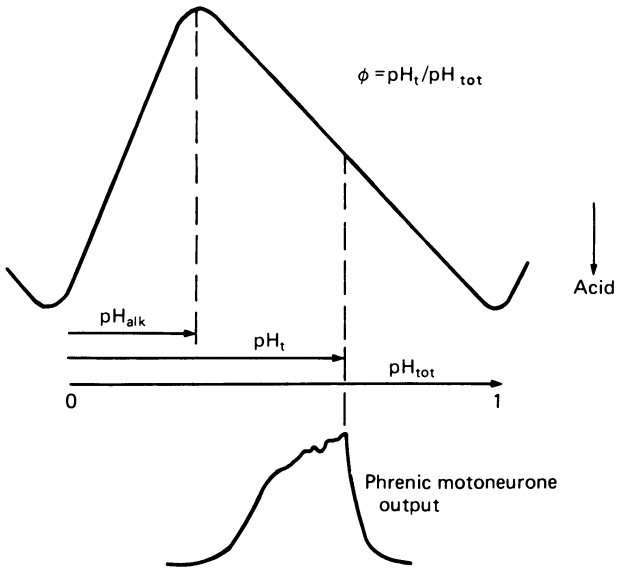

Fig. 4. Illustration of method of measurement of phase (ϕ). pH_{tot} – duration of pH cycle; pH_t – duration of pH cycle up to the point where 'integrated' phrenic activity reaches a peak; pH_{alk} – duration of the upstroke of the oscillation. $\phi = pH_t/pH_{tot}$. The points 0 and 1 were used to define the onset and end of each pH cycle.

than two turning points. An estimate of the variation (*Var*) of the dependent variable (mean value, $\bar{Y}$) with ϕ was calculated from the following expression:

$$Var(\%) = 100(Y_{max} - Y_{min})/\bar{Y}. \quad (1)$$

The values of ϕ were recorded for each sequence where *Phr*, T_i and T_e were maximal and minimal. For each circumstance, values of ϕ were assessed with circular distribution statistics (Zar, 1974) to determine whether the results were concentrated at a particular mean ϕ, or whether the dispersion of ϕ was such that no mean value could be assigned. If a mean value could be defined, the circular S.D. was calculated.

Temperature homeostasis

Body temperature was controlled using a blanket heated by a current proportional to any changes in rectal temperature away from 37 °C (C. F. Palmer Ltd).

RESULTS

General observations

In all animals the mean arterial pressure was over 90 mmHg and the rectal temperature was maintained within 1 °C of 37 °C. In two animals the mean and pulsatile blood flow were measured in the carotid arteries and found to be equal on

TABLE 1. Control values of T_i, T_e, pH_a, P_{a,CO_2} and P_{a,O_2} for the three groups of experiments

		T_i (sec)		T_e (sec)		pH_a		P_{a,CO_2} (mmHg)		P_{a,O_2} (mmHg)	
Experiment	n	mean	range	mean	range	mean	range	mean	range	mean	range
Tidal volume changes	13	2·52	1·07–5·00	2·68	1·07–5·50	7·34	7·26–7·46	41·7	31·4–49·8	90·0	63·4–115·1
Stationary phase shifts	10	2·27	0·77–5·28	2·45	1·60–4·41	7·33	7·24–7·43	47·3	37·7–59·4	86·0	68·0–115·1
Time-varying phase shifts	10	2·59	1·81–3·14	3·79	1·50–7·01	7·35	7·32–7·41	45·1	34·7–50·6	83·3	65·2–106·6

A representative value for each of the measured variables (T_i, T_e, pH_a, P_{a,CO_2} and P_{a,O_2}) was obtained from all dogs at the time of each of the three types of experimental manoeuvre. n refers to the number of dogs in which each type of experiment was performed. The term 'control' does not apply to the values for T_i and T_e of the time-varying phase shift experiments; the values given are the means obtained from the 50–200 breaths of the experimental runs. The long mean T_e of this group is due to two particularly slow-breathing animals.

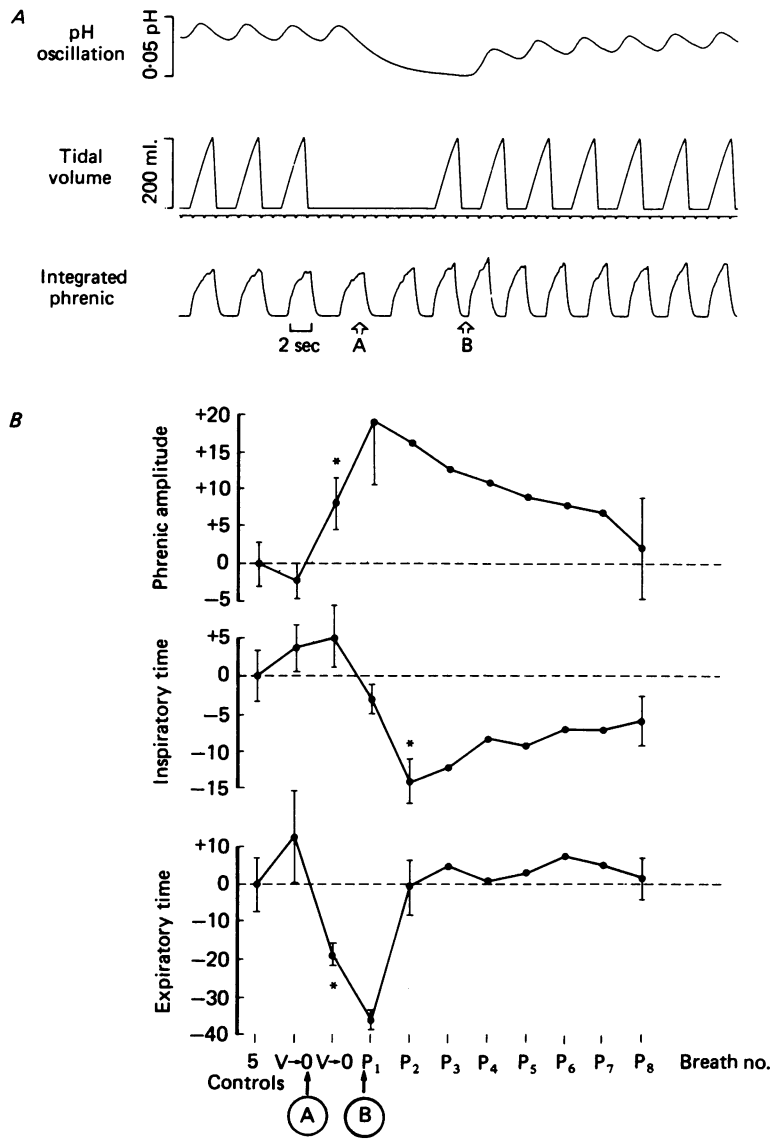

Fig. 5. The effect of withholding lung inflation for two breaths in one dog. In *A* the traces from above downwards are (i) arterial pH, acid direction downwards, (ii) inflation volume, (iii) time trace marked at one sec intervals and (iv) 'integrated' phrenic signal. A is where the pH trace first deviates from its control path and B is where the recovery of pH starts. The lung-pH electrode circulation time can be calculated from the onset of inflation following the apnoeic period to point B; it is approximately 3 sec. $\dot{\Phi}$, mean $\pm$ 1 $\times$ S.D. The results obtained in five consecutive runs are summarized in *B*. The responses of phrenic amplitude (above), inspiratory time (centre) and expiratory time (below) are all expressed as mean percentage changes from the mean of their control values (0); the scales for T_i and T_e are different. In each run, five control breaths were recorded before inflation was withheld (V$\rightarrow$0). The first point on each graph therefore represents the mean of twenty-five control breaths (five per run). P_1 to P_8 refers to the eight recovery breaths. The S.D. about the mean is given where appropriate. A and B are as defined in *A*. Asterisks show the first significant changes from control values.

the two sides and unaffected by the insertion of the loop which housed the pH electrode.

Table 1 shows the mean and range of pH, P_{a,CO_2}, P_{a,O_2}, T_i and T_e for the three experimental procedures, i.e. tidal volume changes, stationary phase shifts and time-varying phase shifts. In two dogs oxygen was added to the inspired air to raise the P_{a,O_2} up to or greater than 100 mmHg. The P_{ET,CO_2} was always less than the P_{a,CO_2}, the difference varying between 0·7 and 15·6 mmHg, but in any one animal the variation in this gradient was found to be under 3 mmHg. P_{ET,CO_2} was used as a guide to the control of P_{a,Co_2}, but never to determine absolute values of P_{a,CO_2}. The large values of T_i reflect the effect of vagotomy on respiratory frequency. In the range of arterial gas tensions and respiratory frequencies shown in Table 1 the mean amplitude of the pH oscillations was 0·028 (range 0·016–0·038 u.). The upstroke of the pH oscillations (pH_{alk}) varied between 0·25 and 0·37 of the total pH cycle (the cycle length being from 0 to 1, see Fig. 4).

In the eleven dogs in which intra-aortic injections of either cyanide or dithionite were given the delay between a signal at the electrode and the onset of a response in the 'integrated' phrenic signal was determined. For potassium cyanide this delay was 1·1 sec (S.D. 0·3) in five dogs and for sodium dithionite it was 1·2 sec (S.D. 0·7) in six dogs.

Tidal volume changes

The effect of transient changes in blood gas composition for one or two breaths was studied in thirteen dogs; typical results are shown in Figs. 5 and 6. A maximal response was seen in the first three experimental breaths but there were no consistent significant effects on *Phr*, T_i and T_e before the carotid arterial pH had changed as a result of the change in tidal volume. In the example in Fig. 5*A*, where ventilation was stopped for the period of two breaths (total of 11 sec), there was an increase in *Phr* and a shortening of both T_i and T_e. The mean results from five such runs in the same animal are shown in Fig. 5*B*. It can be seen that the changes in respiratory motor output closely follow the changes in carotid arterial pH, but that T_e both responded faster and recovered more quickly than *Phr* and T_i.

Changes of a similar magnitude, but in the opposite direction, were seen in response to increases in tidal volume (Fig. 6*A*). Again it should be noted that *Phr* and T_i were slower to respond and recover than T_e (Fig. 6*B*). For both increases in tidal volume and withholding inflation the maximum effect on T_e was seen in the breath preceding or coinciding with point *B* (see Figs. 5 and 6) and the maximum effect on *Phr* and T_i occurred either in the breath which coincided with point *B* or the breath immediately afterwards.

In all series, one or more of the respiratory variables showed a significant response to a change in tidal volume. Table 2 contains all the results; it includes four experiments which differed only in that carotid arterial pH was not recorded. Changes in mean pH of the order of 0·02–0·03 units predominantly affected T_e; a fall in pH resulted in a decrease in T_e and a rise in pH resulted in an increase in T_e. Changes in the opposite direction were seen in *Phr*. Changes in T_i, although frequently different from the control values, were inconsistent in direction; this is reflected in small mean

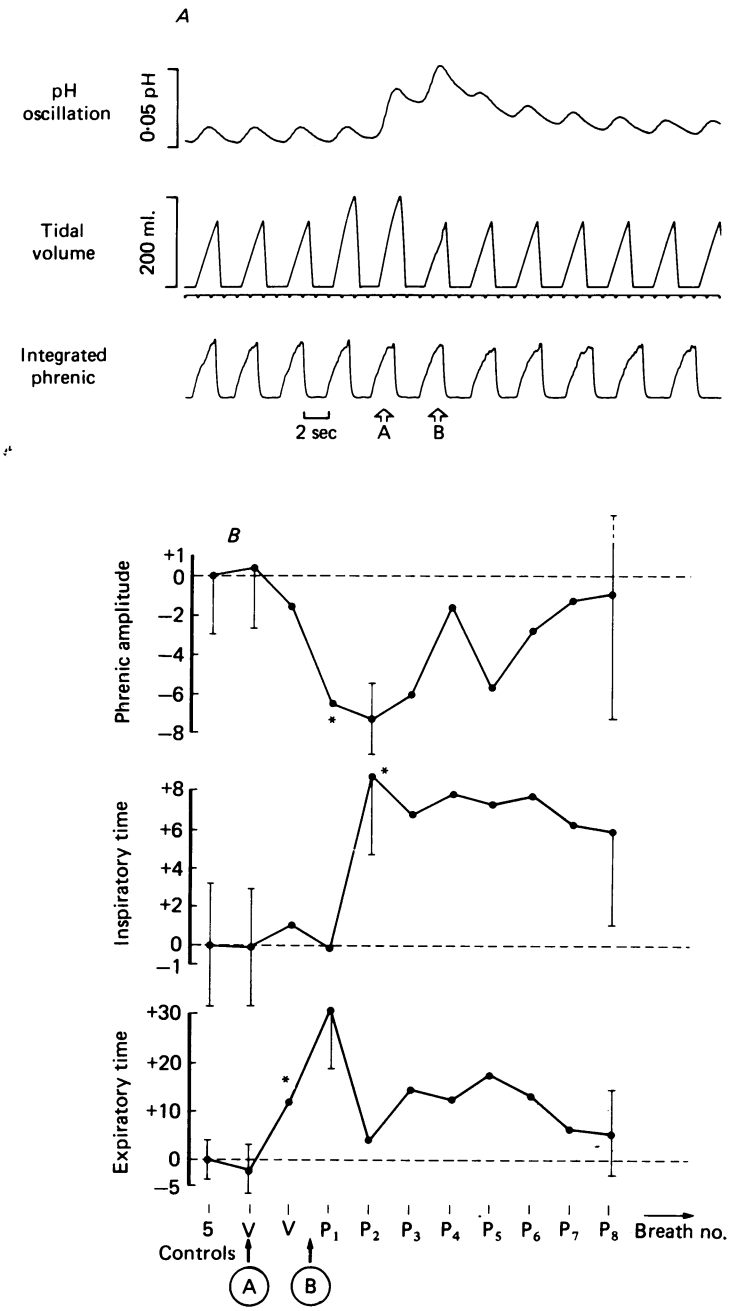

Fig. 6. The effect of increasing tidal volume for two breaths in one animal. In *A* the traces and point A and B are as defined in Fig. 5*A*. In *B* the results obtained in five consecutive runs are summarized. The description of the graphs is as for Fig. 5*B*. V refers to breaths in which inflation was increased by approximately 30 %. Asterisks show the first significant changes from control values. ⧳, mean ± 1 × S.D.

TABLE 2. Effect of increased and withheld tidal volume on respiratory output measured in terms of *Phr*, T_i and T_e, and the effect on the carotid artery pH oscillations

Manoeuvre		Cartoid artery pH								
					ΔPhr%		ΔT_i		ΔT_e%	
Tidal volume change	n	Oscillation amplitude (mean, S.D.)	Maximum change (mean, S.D.)	n	mean %, S.D.	*n*	mean %, S.D.	*n*	mean %, S.D.	*n*
Down	21	0·013, 0·008	−0·020, 0·013	14	+9·4, 4·9	16	−0·7, 12·4	17	−20·3, 10·2	18
Up	5	0·009, 0·005	+0·029, 0·021	4	−4·8, 2·2	5	+5·3, 7·2	3	+18·8, 11·1	4

For the manoeuvres and carotid artery pH recording, n = no. of series. For *Phr*, T_i and T_e, *n* = no. of series in which the measured variable was significantly different from the control values. The results of the changes in *Phr*, T_i and T_e are shown as the maximum response expressed as the percentage of the five control breaths, for those values where the response was significant. The carotid artery pH measurements refer to the nine dogs in which it was carried out. Oscillation amplitude refers to the size of the control pH oscillations and maximum change expresses the difference between the acid trough of the control oscillations and the most acid point resulting from withholding inflation (B in Fig. 5*A*) and the difference between the alkaline peak of the control oscillations and the highest alkaline point due to increase in V_T (B in Fig. 6*A*).

percentage changes and large standard deviations. Reduction of pH caused an increase of T_i in seven series and a decrease in ten series. With increases in tidal volume and resulting increase in pH, there was a significant change in T_i in three series; in two this was an increase and in the third this was a decrease.

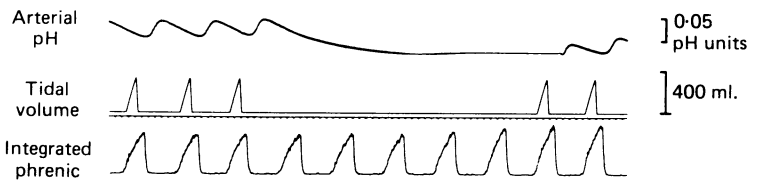

Fig. 7. The effect of withholding lung inflation for five breaths following denervation of the carotid bodies. From above downwards the traces are (i) arterial pH, acid direction downwards, (ii) inflation volume, (iii) time trace marked at one sec intervals and (iv) 'integrated' phrenic signal. The pH trace has reached the limit of the pen scale after the third missed inflation. Note that no changes in the pattern of phrenic motoneurone output can be detected within the first four breaths during the apnoeic period.

The lung-electrode circulation time could be obtained from traces like that shown in Fig. 5*A* by measuring the time from the onset of inflation to the point B where the pH trace began to deflect upwards. In nine dogs the mean time was 3·04 sec, S.D. 0·64 sec.

Effect of denervating the carotid bifurcation

In four dogs denervation abolished the respiratory response to switching off the ventilator for two breaths. However, in two dogs when inflation was withheld for a longer period, a respiratory response was seen after the fourth breath (Fig. 7). At the fifth breath there was a significant change of *Phr*(+21·2%, S.D. 4·9); changes in T_i (5·0%, S.D. 14·0) and in T_e (−20·7%, S.D. 38·7) were neither consistent nor significant.

Stationary phase shifts

Eighty phase changes were made in ten dogs. The criterion described in the methods for inclusion of a phase change in the results was that there was less than a 10% difference between pre- and post-control values for any of the measured variables (i.e. *Phr*, T_i, T_e and ϕ); this criterion was met in forty-nine of the trials. In seventeen of these there were no differences between pre- and post-control values, nor were there any consistent changes in mean P_{ET,CO_2} or mean intra-arterial pH between the pre- and post-control values ($\Delta P_{ET,CO_2}$ −0·3, S.D. 1·0 mmHg; ΔpH +0·002, S.D. 0·013 u.). There were also no consistent changes in P_{ET,CO_2} between the values obtained during the experimental change of phase and the mean of the pre- and post-control values ($\Delta P_{ET,CO_2}$ +0·1, S.D. 1·1 mmHg; ΔpH −0·002, S.D. 0·008 u.).

Initial analysis was carried out on the seventeen trials alone but the addition of the other thirty-two trials in no way altered the interpretation of the results. The following analysis is based therefore on the results of forty-nine trials in ten dogs. The mean values of ϕ, T_i, T_e and *Phr* associated with each change in phase were compared with the mean of the pre- and post-control values.

The scope for varying the phase was limited because the control phase relationship was determined by the animals own respiratory rate and the circulation time between the lungs and the electrode; in most animals the end of inspiration coincided with the downstroke of the pH oscillation (Fig. 2). A single complete phase change consisted of two shifts, one from the pre-control to the experimental state and a second back to the post-control (see Methods). For the convenience of analysis, the effects induced by any phase change from the control state have been divided up

TABLE 3. Effect of changes in phase (ϕ) on T_e

Change in ϕ	ϕ Control mean (range)	ϕ Experimental mean (range)	T_e (sec) Control mean (S.D.)	Number of trials significant	ΔT_e % (S.D.)
Confined to downstroke group A	0·63 (0·4–0·8)	0·53 (0·4–0·9)	2·45 (0·69)	6/20	+0·5 (8·1)
From downstroke to upstroke group B	0·55 (0·4–0·9)	0·22 (0·1–0·4)	2·20 (0·26)	20/20	+17·4 (7·5)
From upstroke to downstroke group C	0·13 (0·1–0·2)	0·54 (0·3–0·9)	3·26 (0·78)	5/9	−28·9 (13·9)

The trials have been divided into three groups; group A where ϕ changes were confined to the downstroke, group B where ϕ changes were from downstroke to upstroke of the pH oscillation and group C where the ϕ change was from upstroke to downstroke. The mean of the pre- and post-control values for ϕ and T_e are shown in the second and fourth columns respectively; included are the range of values for ϕ and the S.D. for T_e. The new ϕ after the phase change is shown in column 3. Column 5 shows the number of trials where the change in phase was significant (first number) as well as the total number of trials (second number). The mean percentage change in T_e and S.D. of the trials that were significant is shown in column 6.

into three groups: group A consisted of those trials where the phase change, although statistically significant, was confined to the downstroke of the pH oscillation; in group B the phase change was from the downstroke to the upstroke of the oscillation and in group C it was the reverse, from the upstroke to downstroke. The results are given in Table 3.

The largest changes occurred in T_e. In group B, it lengthened significantly in all twenty trials by a mean of 17·4%. The reverse phase change (group C) produced a shortening in all nine trials of which five were significant; the mean drop in these five trials was 28·9%, S.D. 13·9. In group A there was no consistent alteration in T_e in the few trials in which the change was significant.

The changes in T_i and *Phr* in group A and B, when significant, were small and not consistent in direction. In group C, *Phr* fell in all nine trials of which six were significant and in these the mean change was −18·8%, S.D. 16·7. There was no consistent change in T_i.

It will be seen that mean T_e during the control periods for group B was 2·2 sec, and for group C it was 3·3 sec ($P < 0·001$). This is consistent with the finding that T_e is longer and respiratory frequency slower when end-inspiration coincides with

the upstroke of the pH oscillation. It is also clear that the results obtained are entirely reversible and do not demonstrate any hysteresis in the system.

Effect of denervating the carotid bifurcation on the response to stationary phase changes

This was studied in two dogs. Before denervation, end-inspiration coincided with the upstroke of the pH oscillation in both dogs and the phase change tested was from upstroke to downstroke. As expected (group C, Table 3) *Phr* fell and T_e shortened

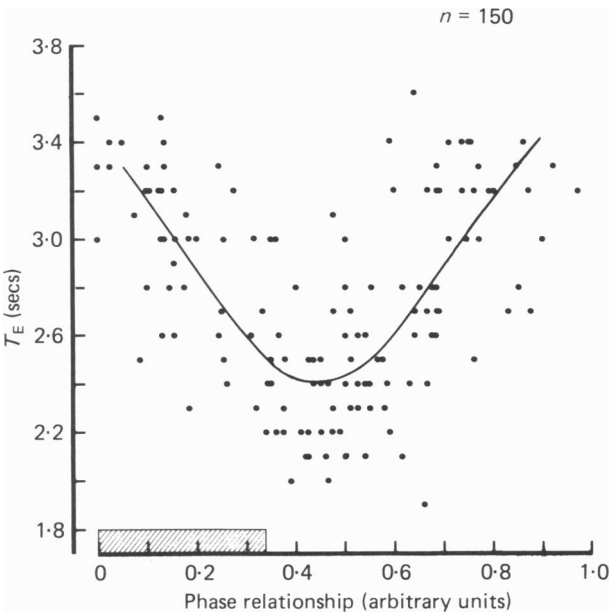

Fig. 8. The relationship between T_e and ϕ determined from a time-varying phase shift experiment in one dog. Each breath is represented by one point (n = 150). The shaded area on the abscissa corresponds to the upstroke of the pH cycle (pH_{alk}). The phase relationship (ϕ) on the abscissa is scaled from 0 to 1 (see text and Fig. 4); 0 and 1 occur at the same position on the pH cycle. Note that T_e varies with ϕ; minimum values occur at ϕ = 0·42 and maximum values occur at ϕ = 0·95.

in all six trials. The fall in *Phr* was significant in five trials (mean −21·8%, S.D. 16·6) and the change in T_e was significant in three trials (mean −38·9%, range −37·9 to −40·0). After denervation, end-inspiration coincided with the downstroke of the pH oscillation in both dogs so that the phase change tested could not be the same as before blockade. The phase change performed on these occasions was from downstroke to upstroke. With the carotid bodies intact, a move from downstroke to upstroke would be expected to increase T_e (Table 3, group B) without any consistent change in T_i or *Phr*. However, following carotid body denervation there were no significant changes in T_e, T_i or *Phr* in any of the four trials.

Time-varying phase shifts

In nine out of ten dogs, 100–200 consecutive breaths were recorded; in the remaining dog the series consisted of fifty breaths. Mean changes in blood gas composition were monitored from (*a*) records of P_{ET,CO_2}, (*b*) measurements of arterial

blood gas composition at the beginning and end of each run, and (c) records of intra-arterial pH. The control values of T_i, T_e and blood gas composition are shown in Table 1. The mean results from all ten dogs showed that during the sequence of breaths there were only small changes in arterial blood gas composition ($\Delta P_{a,O_2}$ +2·9, S.D. 4·2 mmHg; $\Delta P_{a,CO_2}$ −1·9, S.D. 2·3 mmHg; ΔpH −0·004, S.D. 0·005 u.).

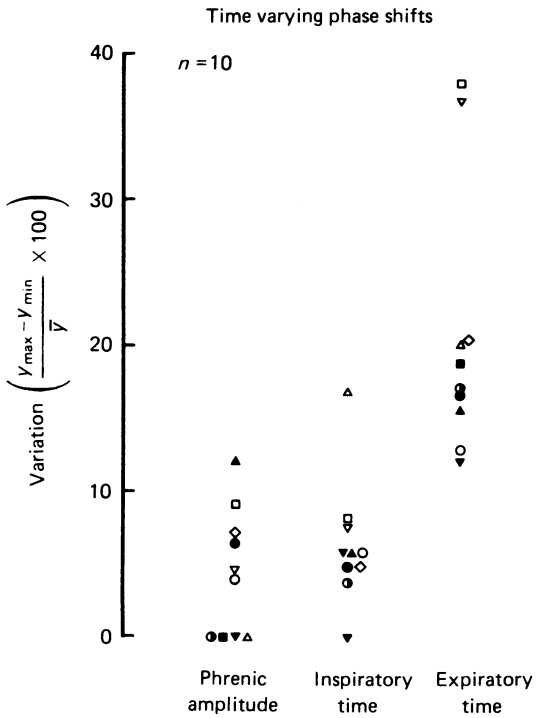

Fig. 9. The percentage variation about the mean values of *Phr*, T_i and T_e resulting from a time-varying phase shift experiment in ten dogs. Each animal is represented by a different symbol.

The respiratory variables, that is *Phr*, T_i and T_e were each plotted against ϕ; an example showing T_e against ϕ is shown in Fig. 8. It can be seen that although there is quite a large scatter in the points, an effect of the phase relationship is present. The fluctuation of the respiratory variables with phase were obtained from the fitted curves (see Methods). Only two definite turning-points were seen, one maximal (Y_{max}) and one minimal (Y_{min}).

T_e showed significant fluctuations in all ten trials by a mean of 21 %. T_i showed significant fluctuations in nine out of the ten trials by a mean of 7 % and *Phr* showed significant fluctuations in six out of the ten trials by a mean of 7 % (Fig. 9).

The phase relationships associated with maximal and minimal values of *Phr*, T_i and T_e are shown in Fig. 10. T_e was maximal when ϕ was 0·21, circular S.D. 0·14 and minimal when ϕ was 0·70, circular S.D. 0·14. T_i was maximal when ϕ was 0·92, circular S.D. 0·16 and minimal when ϕ was 0·44, circular S.D. 0·16. *Phr* was maximal when ϕ was 0·38, circular S.D. 0·15 and minimal when ϕ was 0·88, circular S.D. 0·17. However, no confidence can be attached to the mean for *Phr* because the results could not be distinguished from a uniform circular distribution ($0{\cdot}2 < P < 0{\cdot}3$).

Effect of denervating the carotid bifurcation on time-varying phase shifts

In two dogs, runs of between 50–130 breaths were recorded before (included in Fig. 9) and after denervation. After denervation, there were no significant fluctuations of T_i, *Phr* and T_e with ϕ.

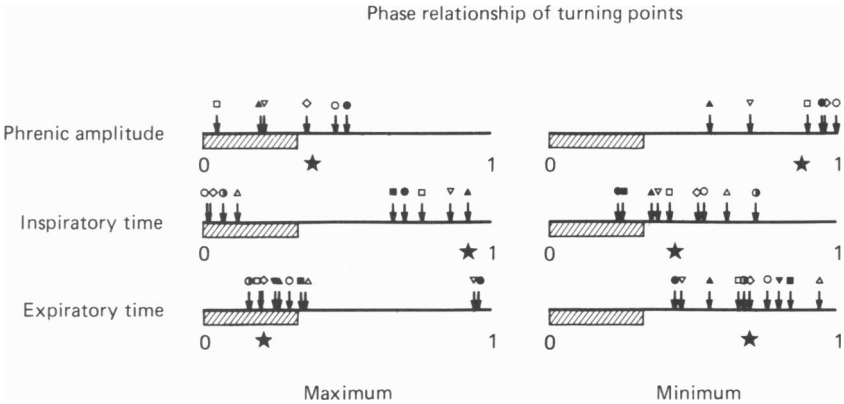

Fig. 10. The relationship between ϕ and maximum and minimum values of *Phr*, T_i and T_e determined from the time-varying phase shift experiment performed in ten dogs. The same symbols as in Fig. 9 have been used to represent individual dogs. Results have been indicated only where the variable fluctuated significantly with ϕ. Each point is shown in relation to the pH cycle depicted as a horizontal line from 0 to 1, as in the abscissa of Fig. 8. Stars represent the position of the mean maximum and minimum values (see text). The hatched areas correspond to the upstroke of the oscillation.

DISCUSSION

These experiments clearly demonstrate that changes in the phase relationship between respiratory and blood gas cycles have a significant effect on respiratory output variables; this effect, which is predominantly on T_e, is independent of changes in mean blood gas composition. In addition, small transient disturbances in the mean level of blood gases in both directions produce changes in ventilation that tend to correct the disturbance. These changes result principally from changes in respiratory rate due to the dominant effect of alteration in T_e. Both these effects are mediated through the chemoreceptors at the carotid bifurcation.

Small changes in mean blood gas composition (typically $\pm$ 0·02–0·03 pH units, corresponding to $\pm$ 2–3 mmHg P_{a,CO_2}) were produced by small perturbations in ventilation such as would occur in normal breathing, for example a short apnoea or a small increase in tidal volume. The respiratory response to these changes was rapid once the disturbance arrived at the carotid body and recovered quickly with little apparent overshoot. This degree of sensitivity is consistent with the earlier work of Fitzgerald, Zajtchuk, Penman & Perkins (1964) who demonstrated that the carotid bodies could respond to a step change of P_{a,CO_2} as small as 2 mmHg. A particular feature of the experiments reported here is that a fall in P_{a,CO_2} (rise in pH) reduced respiratory motor output and this provides confirmation that a drive to breath originating from the peripheral chemoreceptors exists even when the blood gas composition is in the normal range. It is worth noting that the fastest and greatest

response was in T_e. This high sensitivity of T_e to change, induced by each of the experiments reported in this study, lends support to the view that afferent input from the carotid body chemoreceptors can influence T_e independently of T_i and *Phr*. Because of the independence, changes in T_e and *Phr* could have opposing effects when considered in terms of minute ventilation; such an example is seen in group C of the stationary phase shift experiments. The independent effect of *Phr* on T_i and T_e suggest that separate functional mechanisms are involved in the control of the two phases of the respiratory cycle, at least in the vagotomized dog. The findings have some resemblance to those obtained in conscious man when ventilation was stimulated either by alternate breath oscillations of alveolar P_{CO_2} (Drysdale & Ward, 1976), or by steady-state CO_2 inhalation (Gardner, 1977; Cunningham & Gardner, 1977). It may be relevant to note that in conscious man in eupnoea, there is no evidence of any vagal control of breathing (Guz, Noble, Widdicombe, Trenchard, Mushin & Makey, 1966).

The rapid responses in the present set of experiments to transient changes in blood gas composition were abolished by carotid body denervation. However, a delayed response was clearly shown (Fig. 7) presumably mediated through the chemosensitive regions within the brain stem. This slower response was characterized by a larger increase in *Phr* and a smaller change in T_i and T_e than that obtained in the presence of intact carotid bodies. This is consistent with the findings of Borison, Hurst, McCarthy & Rosenstein (1977) where the predominant effect of stimulation of the central chemosensitive areas in the unanaesthetized decerebrate cat was to increase tidal volume with little or no effect on timing.

There is no evidence from our records that changes in mean pH, P_{a,CO_2} or P_{a,CO_2} were responsible for the respiratory effects of a change in phase. The measured changes in mean pH, P_{a,CO_2} and P_{a,O_2} were very small and varied in direction. Indeed, in the stationary phase change experiment, it became evident that the phase changes could override effects due to alterations of mean blood gas composition. Thus when T_e lengthened, P_{ET,CO_2} rose and it would be anticipated from the experiments where tidal volume was changed, that this rise in P_{a,CO_2} would then shorten T_e. This did not occur and the tidal volume of the respirator had to be adjusted to hold mean arterial pH and P_{a,CO_2} constant.

Experiments in man and animals have demonstrated that chemical stimuli can influence inspiration and expiration independently (Drysdale & Ward, 1976; Eldridge, 1976; Gardner, 1977; Cunningham & Gardner, 1977; Ward & Cunningham, 1977). The effects of a change in ϕ in the present experiments on phrenic motoneurone output can satisfactorily be accounted for by relating T_i and *Phr* to the chemical events at the carotid body during inspiration and also by relating T_e to the events occurring during expiration. In order to do this, allowance must be made for the time delay of 1 sec between a change in pH at the electrode and the resulting change in phrenic motoneurone output.

This analysis shows that *Phr* was maximal and T_i was minimal when the acid trough of the oscillation occurred during inspiration; whilst *Phr* was at a minimum and T_i at a maximum when the alkaline peak of the oscillation was associated with inspiration. Likewise T_e was shortest when the trough of the oscillation occurred during expiration and longest when the peak coincided with expiration. Thus, T_i and

T_e are shortened by a fall in pH during inspiration and expiration *respectively*; the opposite effect occurs with rises in pH during inspiration and expiration. Thus a change in ϕ may cause T_I and T_e to change in opposite directions. In the present experiments the dominant effect of T_e will determine respiratory frequency.

It is interesting to note that the results are also compatible with the findings of Mueller, Plaas-Link, Luttman, Mückenhoff & Loeschke (1977) who studied the respiratory response to artificial oscillations in arterial P_{CO_2} induced in both carotid arteries of the cat.

The time-varying phase shift experiments support and amplify earlier studies by Black & Torrance (1971). These authors observed that the total number of impulses, the peak frequency of discharge and the duration of discharge measured in a single phrenic efferent varied slightly with the phase of the ventilation cycle at which the inspiratory discharge began. The duration of T_e was not reported. Black & Torrance (1971) also observed a tendency for the respiratory frequency of the cat to 'lock in' to the frequency of the ventilator. The time-varying phase shifts of the present experiments were not designed to investigate this phenomenon. Nevertheless, the tendency to 'lock in' was observed for short periods in four animals. A similar observation has been made by Mueller *et al.* (1977).

The results of the stationary phase shifts and time-varying phase shifts reported here were at least qualitatively similar. The correspondence between them was gratifying, considering that the method of analysis was entirely different. The two methods are mutually supportive. The stationary phase shift data are limited in their range of study yet without them, it would have been impossible to conclude that the *Phr*, T_I and T_e are dependent on the phase relationship; it could have been argued that the relationship between phrenic motoneurone output with phase was due to consistent variation in phrenic motoneurone output with time, independent of afferent input. The stationary phase shift experiments demonstrate that this is not the case.

Two aspects of our experimental design may have minimized the respiratory effects of changes in phase. First the experiments were conducted with the P_{a,O_2} close to or within the accepted range of normoxia; under hypoxic conditions the effects of chemical stimuli on the carotid body would presumably be enhanced (Lloyd, Jukes & Cunningham, 1958; Bouverot, Flandrois, Puccinelli & Dejours, 1963). Secondly, the placement of the electrode in a loop in one common carotid artery, while having no observable effect on blood flow, must have delayed the humoral signal arriving at the ipsilateral carotid body. The effect of this delay must have blunted the magnitude of the response because of the asynchronous stimulation of the two carotid bodies. This could be of especial importance in view of work showing that the results of stimulating both carotid bodies are multiplicative rather than additive (Eterradosi, Benchetrit, Idelman, Poupot & Lemarchands, 1967).

The present experiments would not have been possible without there being a technique whereby ventilation of the lungs could be separated from the output of the bulbo-pontine generator. Thus the feed-back loop was completely broken when the ventilator was in the 'automatic mode' and partially so in the 'triggered mode'. The effect of changes in phase on respiration could be studied without recourse to interference with the circulation such as occurs in cross-circulation experiments and with the insertion of mixing chambers and delay coils. The price paid, however,

for using this technique was that vagotomy had to be performed; otherwise volume feed-back would have completely overridden any change due to chemical feedback (Cross & Guz, 1976*a*, *b*; Cross, 1978). The disadvantage of vagotomy is, of course, that it is unphysiological; the connexion of the aortic chemoreceptors has been cut, whilst respiration tends to be slower and more irregular. The importance of these disadvantages may be less than appears at first sight. Comroe & Mortimer (1965) showed that the hyperpnoea from injections of cyanide in dogs was always greater from stimulation of the carotid bodies than from the aortic bodies; this suggested that the carotid bodies were of much greater importance than the aortic bodies in ventilatory control in the dog. Whilst T_i in the present experiments was on average longer than would be encountered in the non-vagotomized state, it was found that during the delay of 40 min or longer between section of the vagi and the start of the experiments, the respiratory rate increased and the irregularity of breathing was considerably reduced. The result was that a wide range of respiratory rates were seen, some of which were in the lower part of the normal range (Table 1). Finally, the results may be of great relevance to anaesthetized or unanaesthetized man, where there appears to be no influence of lung volume feed-back on the pattern of breathing during eupnoea (Guz, Noble, Trenchard, Cochrane & Mackey, 1964; Guz *et al.* 1966).

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